Sports as a cause of oxidative stress and hemolysis

Javier F. Bonilla, M.D.[1], Raúl Narváez, M.D., M.Sc.[2], Lilian Chuaire, M.Sc.[3]

SUMMARY

More than three decades ago, it was established that anemia, a cause of tissue oxygenation deficiency, can be caused by exercise. However, this preliminary relationship really corresponds to an event where the plasma is diluted and for this reason the term «sports pseudoanemia» was made. New data relate exercise from moderated to exhaustive, with blood loss through gastrointestinal and urinary tracts, as well as erythrocytes rupture by mechanical, osmotic and oxidative events. Therefore, now the association between chronic exercise and impairment in erythrocytes number and form is clearer, which is evidence in favor of a true anemia in sports. In this anemia it is evident the ferropenic etiology. But recent information opens discussion about whether hemolytic etiology is a co adjuvant factor to anemia, and on the role of oxidative stress in it. This paper is an updated review for a relationship between sports and anemia, and for assessing causes of ferropenic anemia and for sports hemolysis.

Key words: *Anemia; Physiology; Exercise.*

El deporte como causa de estrés oxidativo y hemólisis

RESUMEN

Desde hace más de tres décadas se estableció que el ejercicio puede producir la anemia, una de las causas en la deficiencia de oxigenación a los tejidos. Sin embargo, esta relación preliminar realmente corresponde a un evento donde el plasma se diluye, razón por la cual no es una verdadera anemia, por lo que se acuñó el término "pseudoanemia del deportista". La nueva información relaciona el ejercicio, de moderado a exhaustivo, con la pérdida de sangre a través de los sistemas gastrointestinal y urinario, así como con la ruptura de los eritrocitos debida a eventos mecánicos, osmóticos y oxidativos. Entonces ahora es más clara la asociación entre el ejercicio crónico y el deterioro en el número y forma de los hematíes, lo que constituye evidencia en favor de una verdadera anemia del deportista, de clara causa ferropénica. La información reciente abre la discusión acerca de la etiología hemolítica como factor coadyuvante en la anemia, y acerca del papel que en ella tiene el estrés oxidativo. La presente es una revisión actualizada que relaciona el deporte y la anemia, además de presentar los orígenes de la anemia ferropénica y de la hemólisis en deportistas.

Palabras clave: *Anemia; Fisiología; Ejercicio.*

Anemia could be defined as the status in which the quantity and quality of circulating erythrocytes is below the normal levels for a determined individual, according with the reference ranges for hemoglobin (Hb) and erythrocyte count appropriate to age, sex and sea level as well.

There is a recent number of investigations that inform about changes in the physiological erythrocyte indices as well as in the erythropoyesis itself after a physical training session in general, or a high intensity aerobic exercise[1]. This fact leads to postulate the physical exercise as a possible cause for anemia. From this it was derived more than three decades ago the term «sportsmen anemia»[2-4] to define a limit anemic status (borderline) proper for individuals who practice some physical activity on a regular basis, e.g. athletes[5]. Who were found a hemodilutional effect, which should not be considered as a true anemic condition but a reologycal adaptation to exercise[6].

Several researches indicate that the frequency of this kind of anemia is similar in problem groups constituted by athletes, in respect to control groups. Exercise could affect Hb concentration in an undetermined way, since during and after the exercise session it is possible to find

1. Assistant Professor, School of Rehabilitation and Human Development, Universidad del Rosario, Bogotá, Colombia. e-mail: jfbonill@urosario.edu.co
2. Principal Professor, Physiology Laboratory Coordinator, Basic Sciences Institute, School of Medicine, Universidad del Rosario, Bogotá, Colombia. e-mail: rnarvaez@urosario.edu.co
3. Principal Professor, Basic Sciences Institute, School of Medicine, Universidad del Rosario, Bogotá, Colombia. e-mail**:** lchuaire@urosario.edu.co
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modifications in its values, for example due to hemoconcentration or to changes in the individual hydration grade[7].

A great part of the difficulty to precisely determine whether there is an anemia due to exercise resides in the existing differences among the researched population, as well as in the diversity of definitions and etiologies proposed for the anemia[8].

When etiology is associated to dilution, we are not dealing with a true anemia. From this the term pseudoanemia is derived. Today, when the exercise benefits are every time more controversial (vgr. sportsman sudden death[9]) and there exist diverse research in favor or against the physical activity whether it is regular or occasional[10], it is important to determine whether exercise is the cause of anemia in the individual, who could be through other etiologies added to the event associated to dilution. From this perspective, the diagnose for a true anemia must be done through the evaluation of clinical aspects but also hematological parameters such as the media corpuscular hemoglobin (CHCM) which is not affected by the hemodilution[11].

Whenever the ferropenic etiology is evident, exercise is described as one of the causes of anemia. It is under discussion how much the hemolitical etiology contributes to the sports anemia and therefore the role of the oxidative stress in this anemia is being better understood.

Ferropenic anemia secondary to exercise. Ferropenic anemia is related to a decrease in the sportsmen performance[12]. It affects particularly to marathonists and this is the most researched form of anemia[13]. Its causes could be the hemoglobinuria, hematuria, gastrointestinal blood loss and iron loss due to profuse sweat[14].

Hemoglobinuria. The first report about the hemoglobinuria associated to exercise dates from 1881, when Fleischer *in* Jones and Newhouse[15] described the presence of obscure urine in a young soldier after his participation in a march, and he named it as the marching hemoglobinuria. Hemoglobinuria, sometimes associated to hematuria, could promote an anemic condition in competitive athletes[15] specially those running long distances[16]. This anemic condition has been related to hemolysis associated to exercise and to consequent hypohaptoglobinemia and plasmatic Hb increase[17]. There is much evidence that hemoglobinuria could be most common than believed, although it seems to be self-limited and benign[18].

Hematuria. Hematuria is documented from contact exercise (football or box) as well as in non-contact ones (swimming or soaking). It could be macro or microscopic. It is frequent, self-limited and benign, since it disappears 48 to 72 hours after exercise[18]. It could be related or not to gall bladder and/or renal trauma. Whenever it is not traumatic, it is associated with glomerular ischemia due to the constriction of the renal and splenic vessels or it could also be due to an increase in the filtration pressure secondary to the efferent arterioles constriction. The severity of the hematuria is proportional to the intensity and duration of exercise[19] and could course with dehydration, myoglobinuria and lipid peroxidation in erythrocytes[15, 20].

Gastrointestinal blood loss. The digestive blood loss is frequent after a prolonged exercise[21]. In marathon athletes, it is present with a frequency of 8% to 30%, not associated to inflammation nor with gastric blood loss[22] and apparently it is independent from age, career time, abdominal symptoms, and recent ingestion of vitamin C or acetylsalicylic acid[23]. The digestive blood loss related to the intensity of exercise could induce a decrease in the circulating erythrocytes and therefore increase the iron loss[24].

Iron loss for profuse sweat. This form of iron loss has been evaluated in several researches, during and after an exercise session, in trained individuals and non-trained ones as well. Results indicate that this loss depends directly on the amount of sweat, since this is higher in prolonged exercise under high temperatures. There is not a significant difference between women and men. The possible severity of this loss depends on the sportsmen iron reserve (iron status)[25,26].

DOES HEMOLYSIS CONTRIBUTE TO SPORTS ANEMIA?

Several authors have described a significant increase in the destruction of the erythrocytes after intense physical exercise[27]. In 1943 Gilligan *et al.*[17] evaluated the hemolysis associated with intense exercise when determined the plasmatic hemoglobinemia and the hemoglobinuria in marathon athletes. The most affected with this condition are athletes, specially those elite athletes who apparently constitute the most susceptible population. The hemolysis intensity depends on the race distance[27]. Also it has been found hemolysis associated with sports such as swimming[28], soaking, triathlon and aerobic dance[29] as well as in non-competitive races and in rigorous military training[30]. One on the causes for this hemolysis is the fact that after a strong exercise the erythrocytes are more susceptible to

stress, whether of mechanical, oxidative or osmotic type[31]. The oxidative stress could also alter the ionic homeostasis and facilitate the cellular dehydration. These changes decrease the deformability of the red cell thus impeding its passing through the micro-circulation[32].

Telford *et al.*[33] informed about the large ranges in the increase of the plasmatic Hb concentration and the haptoglobine (Hp) decrease in amateur athletes and cyclists who were taken to the maximum oxygen (VO_{2max}) consumption and to the same exercise intensity as well. These facts lead to assume the occurrence of hemolysis in both sportsmen groups. The free Hb increases up to 85+35 Hb mg per each plasma liter, with a higher and a more persistent increase in the Hb plasmatic concentration in the athletes.

On the other side, recent researches suggest the possible hemolysis in sportsmen[34] caused by mechanical effects since they strike erythrocytes and promotes their destruction. The same occurs with long-distance runners when hemolysis occurs as a consequence of the repeated foot impact (footstrike) over the surface[33].

What is the reason why some sportsmen present a higher grade of hemolysis than other, considering that they are under the same conditions of intensity and exercise length? It is necessary to think that hemolysis during and after exercise could be the result of running long distances where erythrocytes are stroke, but it also result from other mechanisms such as the oxidative stress[28, 33].

Sportsmen hemolysis caused by oxidative stress. Oxidative stress is described as the event in which the free radicals are over the systemic mechanisms of the antioxidative defense[35]. In 1978 Dillard *et al.* [36] were the first in demonstrating that physical exercise leads to a lipid peroxidation increase.

It is estimated that at rest, 2% to 5% of electrons flow of the respiratory chain escapes to form reactive oxygen species[37] (ROS), such as peroxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl (OH^-) and those associated with nitric oxide (NO)[38].

The mitochondria is a source of ROS, although it is not necessarily the most important (at least *in vitro*) since during exercise it increases the O_2 tissue consumption range. There is an experimental indicating evidence of increase in the ROS production, as well as oxidative stress and tissue damage associated with exercise, whether exhaustive and severe[39], or moderate[40]. During exhaustive exercise, the muscle oxygen consumption increases 100 to 200 times if compared to the one under rest status[41]. This induces an electron flow increase through the mitochondrial respiratory chain, which at the same time results in an increase of ROS production[38]. It has been determined recently that mitochondrion also generate NO, which could be a part of the free radicals total production during exercise. When NO reacts with O_2, it forms peroxynitrite ($ONOO^-$), a powerful oxidant. This reaction is believed as the main via to generate reactive nitrogen species (RNS)[42].

Oxidative stress could occur in individuals whether or not adapted to exercise, thus making them susceptible to present injury in their enzymatic systems, as well as in lipids and membrane receptors and also in their ADN[42, 43].

Now, the ROS and RNS actions could occur at the end of the exercise session or hours after it. Available information associates exercise with ROS and RNS production through three evidences related between them, such as:

1. The free radicals production is muscle, liver, heart and blood.
2. The increase in the biomarkers of oxidative damage, such as protein carbonyls and substances reactive to thiobarbituric acid[44], and the increase in the exhaled pentane levels, which is a possible result of the lipid oxidative damage[36].
3. The decrease in the antioxidant enzymatic and non-enzymatic levels in heart, blood[45], brain and muscle[46].

Another generating source of ROS is the xanthine-oxidase (XO) via which contributes to the H_2O_2 tissue generation with high xanthine and hypoxanthine concentrations. Tissue hypoxia, through the XO[43] via could generate oxidative stress during exercise[47]. This occurs also after events of ischemia-reperfusion in organs such as heart[48].

XO activation is produced during exhausting exercise thus allowing ROS generation in different tissues[42, 49]. For example, in the skeletal muscle the hypo-xanthine is liberated to blood, thus the XO enzyme is activated[50]. Radak *et al.* [51] demonstrated that the XO via is also committed in the O_2 generation.

The third source of ROS is the peroxisomes. In physiological conditions these organelles produce H_2O_2 but not peroxide. Peroxisomal oxidation of the fat acids is an important source of H_2O_2. Since fat acids are a source of energy for heart and for skeletal muscle during exhaustive exercise, it is probable that peroxisomes contributes to the oxidative stress in sportsmen[38].

A fourth source of ROS is the polymorphonuclears leukocytes (PMN). When neutrophile PMN are activated *(respiratory burst)* they liberate O_2^-. Therefore, if does exist

tissue damage caused by exhaustive exercise, the subsequent neutrophile activation becomes a source of ROS[38, 52]. These activated cells could cause lipid peroxidation in closer cells, and in erythrocytes[53], since their products are able to cross the cellular membrane and produce Hb oxidation[54] which will initiate the hemolysis process. Moreover, the ROS oxidizing action over low density lipoproteins (LDL)[56] and over the lipids of the erythrocyte membrane are associated with hemolysis[53, 57].

The neutrophile PMN could infiltrate the muscle tissue damaged by high-density exercise. When this occurs, the O_2^- generated through oxidase NADPH associated with the membrane, reacts and leads to H_2O_2 formation. This last has become a hipochlorosus acid (HOCl) for a hemoproteic myeloperoxidase secreted by neutrophiles and monocytes. HOCl is an inflammatory mediator, powerful oxidant and chlorinate, since at the same time it generates other reactive metabolites such as nitryle chloride (NO_2Cl) in presence of nitrite. Nitrite could become, through the myeloperoxidase and H_2O_2, the radical nitrogen dioxide (NO_2) that facilitates the formation of other high injuring substances[42]. Since neutrophiles infiltration in the tissue injured by exercise is secondary to production and liberation of proinflammatories, this via may not be the first source of ROS production during exercise. However, it could certainly serve as an important source during the recovery period after exhaustive exercise[58]. A fifth source of ROS is the catecholamines, although their contribution to the free radicals has not been quantified[38]. For example, it has been proposed that in oxidative lesion of the myocardial ischemia-reperfusion, it occurs the epinephrine autooxidation in adrenochrome, associated with O_2 formation.

There has been established that the iron and the hemo group of hemoglobin and myoglobin are potential sources of ROS[42], but it is not clear yet how much they participate in the oxidative stress during or after exhaustive exercise[59].

Several researches *in vitro* discard mitochondria as the main producer of ROS during exercise, since they sustain that these Hb-Mb system is not only capable to generate it but also to increase the reactivity of those produced by other via. Within the radicals generated there is O_2^-, ferryl iron ($Fe^{+4}=O_2^-$) and free radicals joined with proteins[59].

The Hb-Mb system causes injuries in different ways. Thus, following Hb liberation to intravascular space, as a consequence of hemolysis, there is the formation of Hp/Hb complex. But an intense hemolysis saturates the Hp capability to alloy Hb, which takes Hb to remain free in plasma[60]. In the same way, Mb could be free in plasma due to processes such as the rhabdomyolysis, usually associated with exhaustive exercise. When free Hb and Mb are oxidized, they become citotoxic substances and could injury the endothelia (atherosclerosis, vasculitis) and also the erythrocyte itself (intravascular hemolysis)[61].

Hb and Mb oxidation is associated with the ROS liberated from activated leukocytes, during exhaustive exercise and hypoxia. The methemyoglobin (metHb) and metmyoglobin (metMb) thus generated, as well as their derivatives are capable to produce more ROS, besides lipid peroxidation, with formation of hydroperoxides[59,62]. Other researchers have found that hemo group is related with membrane protein oxidation and with formation of surface antigens in senescent red blood cells[63].

Therefore there are established direct and indirect injury mechanisms from Hb and Mb and from their derivatives. One example of the direct one is the primary cytolysis caused by ROS from the type ferryl iron. As an example of indirect mechanisms is the sensibilization to the damage caused by hydroperoxides from the oxidizing LDL type. These mechanisms receive feedback in a way that origins vicious circles: the exercise is a hypoxemic process that generates hemolysis and thus liberates Hb and Mb and their derivatives, which facilitates more hemolysis and more hypoxemia[59].

There exist two control ways that limit the action of the free hemo: the cellular via in which Hp and hemopexin take part and the intracellular one, where hemooxygenase and ferritin participate. These ways are rebased with a defect in the control ways, or if there is an excessive elevation of the free hemo[61].

CONCLUSIONS

Sportsmen pseudoanemia is related with a plasma expansion. In individuals who practice frequent aerobic sport activity it could coexist associated events such hematuria, gastrointestinal blood loss, as well as an increase in the intravascular hemolysis. These factors link exercise with the deterioration of corporal iron reserve and the erythrocyte number and morphology. Likewise there are foreseen much more etiological possibilities not only of entities like anemia, but of a great number of other diseases related to exhaustive and competitive exercise with damaging responses to the organism.

It is necessary to deeply study the reasons why some

sportsmen present higher grades of hemolysis than others, even when they are submitted to similar conditions of intensity and work terms. For this, it must be considered that hemolysis in exercise could result not only from running long distances where erythrocytes are stroke, but also from other mechanisms such as the oxidative stress. To thoroughly understand the mechanisms of action of the oxidative stress and the mechanisms of response of the erythrocyte constitutes an important challenge within the sports physiological field.

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